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# Nonresponse to Hepatitis B Vaccines and the Kinetics of Anti-HBs Production

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No empirical data are available for the anti-HBs titre required for protection against infection with the hepatitis B virus (HBV), but nonresponders to hepatitis B vaccines remain susceptible to infection. There may be an unexplained qualitative difference between hyporesponders and true nonresponders, but there is a clear association with HLA haplotypes. There is no evidence for silent infection with HBV in nonresponders.



**KEY WORDS:** Hepatitis B, vaccines, nonresponse

## INTRODUCTION

Systematic vaccination of individuals at risk of exposure to hepatitis B virus (HBV) has been the main method of controlling the morbidity and mortality associated with hepatitis B infection. The first hepatitis B vaccine was manufactured by the purification and inactivation of hepatitis B surface antigen (HBsAg) obtained from the plasma of chronic HBV carriers [Szmuness et al., 1981; Hadler et al., 1986; Jilg et al., 1988]. This was soon followed by the production of HBsAg using recombinant DNA techniques and expression of the S antigen component in yeast cells.

All studies of the antibody response to currently licensed plasma-derived hepatitis B vaccines and hepatitis B vaccines prepared by recombinant DNA technology have shown that between 5% and 10% or more of healthy immunocompetent subjects do not mount an antibody response (anti-HBs) to the surface antigen component (HBsAg) present in these preparations (nonresponders) or that they respond poorly (hyporesponders) [Dienstag et al., 1984; Craven et al., 1986; Westmoreland et al., 1990; Wood et al., 1993]. The exact proportion depends partly on the definition of nonresponsiveness or hyporesponsiveness, generally less than 10 IU/L or 100 IU/L of anti-HBs, respectively, against an international antibody standard.

Nonresponders remain susceptible to infection with HBV. While several factors are known to affect adversely the antibody response to HBsAg including the site and route of injection, gender, advancing age, body mass (overweight), immunosuppression and immunodeficiency, and mechanisms underlying nonresponsiveness to the S component of HBsAg in humans remain largely unexplained, evidence is accumulating that there is an association between different HLA-DR alleles and specific low responsiveness in different ethnic populations. Considerable experimental evidence is available to suggest that the ability to produce antibody in response to specific protein antigens is controlled by dominant autosomal class II genes of the major histocompatibility complex (MHC) in the murine model [reviewed in Alper et al., 1989; Milich, 1991; Kruskall et al., 1992]. Much effort has been devoted to overcoming class II-linked nonresponsiveness to current hepatitis B vaccine [e.g., Milich et al., 1985a, 1986; Arif et al., 1988].

There is evidence that the pre-S1 and pre-S2 domains have an important immunogenic role in augmenting anti-HBs responses, preventing the attachment of the virus to hepatocytes and eliciting antibodies which are effective in viral clearance, stimulating cellular immune responses, and circumventing genetic nonresponsiveness to the S antigen [Milich et al., 1985a; Klinkert et al., 1986; Alberti et al., 1988; Gerlich et al., 1990]. Thus, a number of studies indicated that the inclusion of pre-S components in recombinant or future synthetic vaccines should be developed. For example, the pre-S2 region is more immunogenic at the T and B-cell levels than the S regions in the mouse model [Milich et al., 1985a,b], as is the case with pre-S1 in the mouse [Milich et al., 1986) and in humans (Ferrari et al. 1992], and circumvents S region nonresponsiveness at the level of antibody production.

Indeed, Milich et al. [1986] demonstrated in the murine model that the independence of MHC-linked gene regulation of immune responses to pre-S1, pre-S2, and S regions of HBsAg would assure fewer genetic nonresponders to a vaccine containing all three antigenic

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regions. Studies conducted in humans with experimental recombinant hepatitis B vaccines containing all three S components of the viral envelope polypeptides demonstrated the enhanced immunogenicity of such preparations when compared with conventional yeast-derived vaccines [Yap et al., 1992, 1995; Shouval et al., 1994; Zuckerman et al., 1996], although several earlier studies with vaccines containing the S and pre-S components revealed significant differences from preparations containing only the S antigen [Marescot et al., 1989; Ferrari et al., 1992; Clements et al., 1994; Suzuki et al., 1994].

No empirical data are available for the anti-HBs titre required for protection against particular routes of infection or the size of the infectious inoculum. The minimum protective level following immunisation has been set in earlier protective efficacy studies at 10 IU/L or more of anti-HBs [Szmuness et al., 1981; Francis et al., 1982]. In both studies most cases of HBV infection occurred in subjects who mounted little or no anti-HBs response. Specifically, a protective level of anti-HBs was defined as 10 IU/L against an international standard [Stevens et al., 1984a; Centers for Disease Control, 1987]. Various studies have also demonstrated that the risk of HBV infection increases as anti-HBs levels decline to 10 IU/L [Stevens et al., 1984b; Coursaget et al., 1986; Hadler et al., 1986; Taylor and Stevens, 1988]. For example, Hadler et al. [1986] reported in a follow-up study of vaccinated homosexual men an overall incidence of HBV infection of 2.9 per 100 person-years with nearly 75% occurring in subjects with anti-HBs titres <10 IU/L at the time of infection and in only a few with anti-HBs titres >50 IU/L. A lower and asymptomatic infection rate of 0.8 per 100 person-years was observed after immunisation of health care workers in nephrology units who had antibody titres of <50 IU/L [Couroucé et al., 1988].

## THE KINETICS OF ANTI-HBs RESPONSE

The titre of vaccine-induced anti-HBs declines, often rapidly, during the months and years following immunisation. The highest anti-HBs titres are generally observed 1 month after booster vaccination followed by rapid decline during the next 12 months and thereafter more slowly [see for example Hilleman, 1984; Jilg et al., 1984; Ambrosch et al., 1987; Gibas et al., 1988; Nommensen et al., 1989; Wiseman et al., 1989]. Mathematical models were designed and an equation was derived consisting of several exponential terms with different half-life periods. It is considered by some researchers that the decline of anti-HBs concentration in an immunised subject can be predicted accurately by such antibody kinetics and preliminary recommendations before the next booster vaccination [Jilg et al., 1984; Fagan et al., 1984a,b; Ambrosch et al., 1987; Nommensen et al., 1989]. If the minimum protective level is accepted at 10 IU/L, which is being debated, consideration should be given to the diversity of the individual immune response and the decrease in levels of anti-HBs as well as to possible errors in quantitative anti-HBs determinations. It would then be reasonable to define a level of >10 IU/L and <100 IU/L as an indication for booster immunisation. It has been demonstrated that a booster inoculation results in a rapid increase in anti-HBs titres within 4 days [Jilg et al., 1988]. However, even this time delay might permit infection of hepatocytes [Nommensen et al., 1989].

Several options are therefore under consideration for maintaining protective immunity against hepatitis B infection:

1. Relying upon immunological memory to protect against clinical infection and its complications [Centers for Disease Control, 1991; Tilzey, 1995], a view which is supported by in vitro studies showing immunological memory for HBsAg in B cells derived from vaccinated subjects who have lost their anti-HBs, but not in B cells from nonresponders [van Hattum et al., 1991], and indeed one cannot recall what has never been memorised [McIntyre, 1995].

2. Providing booster vaccination to all vaccinated subjects at regular intervals without determination of anti-HBs. This option is not supported by a number of investigators because nonresponders must be detected [McIntyre, 1995; Tedder et al., 1993]. Also, while an anti-HBs titre of about 10 IU/L may in theory be protective, this level is not protective from a laboratory point of view since many serum samples may give nonspecific reactions at this antibody level [Westmoreland et al., 1990; Tedder et al., 1993].

3. Testing anti-HBs levels 1 month after the first booster and administering the next booster before the minimum protective level is reached, which is the preferred option. A protective level of 100 IU/L seems to be appropriate.

## REACTIVATION IN NONRESPONDERS

There are numerous reports in the literature that the administration of four, five, or six or more doses of vaccine in apparent nonresponders or hyporesponders resulted in the production of anti-HBs in as many as 50% [Craven et al., 1986; Fagan et al., 1987b; Pasko et al., 1990; Westmoreland et al., 1990], although most reports concern a small number of subjects. A study of some 26 hyporesponders and nonresponders in The Netherlands [Wismans et al., 1988] revealed that some developed anti-HBs after up to a further six inoculations while others failed to respond, leading to a comment that there is an (unexplained) qualitative difference between hyporesponders and "real" nonresponders. Several other explanations had been offered, apart from the administration of the vaccine into an inappropriate site such as the gluteal muscles or intradermally or improper storage of the vaccine. A "slow response" has been considered, as well as modulation of the immune response by genetic factors particularly in relation to HLA type, nonspecific cellular immune defects, concurrent infections, and preferential stimulation of T-suppressor cells.

## HLA HAPLOTYPES IN NONRESPONDERS TO HEPATITIS B VACCINE AND IN RESPONSE TO (HEP B-3) A NEW S, PRE-S1, AND PRE-S2 VACCINE

The HLA class I and class II alleles were determined by McDermott et al. [1996] in the 86 vaccine nonresponders participating in a study of the Hep B-3 vaccine [Zuckerman et al., 1996] by a lymphomicrocytotoxicity technique for class I HLA typing using commercially available plates (Biotest, Germany), and by polymerase chain reaction (PCR) for class II HLA typing. A control group of 115 subjects who were hepatitis B vaccine responders and an additional group of 125 Caucasian new volunteer donors at the Anthony Nolan Bone Marrow Trust were included as further control subjects.

A significant association was found between the HLA phenotype B44;DRB1*0701; DQB1*0201 and the vaccine nonresponse ($P = 0.02$). Those with the phenotype B44; DRB1*0701; DQB1*0201 are nearly four times more likely to be antibody nonresponders when compared to hepatitis B vaccine responders ($P = 0.01$). Previous studies have identified the association of the extended haplotypes B44; DR7; FC31; and B8; DR3; SC01 based on six of nine individuals who were nonresponders to the S vaccine. The correlation of B44; DRB1*0701; DQB1*0201 with hepatitis B vaccine nonresponse was confirmed in the present study representing more accurately the frequency of HLA in the Caucasian population. Despite this, we could not identify a significant association with the HLA phenotype B8; DR3 with nonresponse to HBsAg vaccination as described previously. The frequency of the HLA allele DQB1*0201 was high in our nonresponder population ($P = 0.002$). This allele is in strong linkage disequilibrium with HLA alleles DRB1*0701 and DRB1*0301 which are found in 85% of vaccine nonresponders. The HLA alleles DRB1*1501 and DRB1*0101/02/03 were found at a lower frequency in the nonresponders when compared to the HLA and vaccine control groups, respectively, but were not significant when adjusted for the number of tests carried out. The DR1 antigen has been described previously by less stringent analysis [Walker et al., 1981]. Interestingly, the HLA allele DQB1*0602 was represented at a significantly low frequency in the nonresponder population ($P = 0.02$). This allele is in linkage with DRB1*1501, suggesting that there was an association between the molecular subtypes of DR1 and the response to hepatitis B vaccination.

In summary, a high frequency of HLA class II allele DRB1*0701 and the phenotype B44; DRB1*0701; DQB1*0201 was found in nonresponders compared to controls [McDermott et al., 1996]. There were low frequencies of DRB1*1501, DQB1*0602, and phenotype DRB1*1501; DQB1*0602 among nonresponders. All the initial nonresponders expressing the phenotype B7; DRB1*1501; DQB1*0602 responded to the new vaccine with antibody titres >100 IU/L. The majority of those who failed to mount an antibody response expressed the phenotypes B8; DRB1*0301; DQB1*0201 or B44; DRB1*0701; DQB1*0201 [McDermott et al., 1996].

Nonresponse to the additional pre-S1 and pre-S2 components in the vaccine may be due to dysfunctional interaction of specific HLA molecules with these antigens, lack of T-cell activation, or deletion of specific T cells during thymic education. Further study and typing for additional polymorphism within the HLA-mediated immune response may provide an insight into the mechanism of failure to respond to immunisation against hepatitis B [McDermott et al., 1996]. However, it should be noted that immunogenetic analysis confirmed that an initially distinct group of nonresponders was indeed included in the study of the new pre-S1, pre-S2, S vaccine.

## NONRESPONDERS AND SUSCEPTIBILITY TO INFECTION WITH HBV

Whilst it is accepted that about 5–15% of fully immunocompetent healthy individuals do not mount a humoral antibody response to currently available hepatitis B vaccines, and others are poor responders, there is little in the more recent literature based on long-term follow-up to address the issue of whether such persons are susceptible to infection with HBV. The kinetics of antibody and the issue of post-vaccination testing have been discussed above, and it is undoubtedly of importance for health care workers, their patients, and their employers (under current UK Department of Health Guidelines) to be aware of their protection or lack of protection after immunisation. Equal reliance on an adequate protective cell-mediated immune response (which in the vast majority has not been measured) or primed (or otherwise) immunological memory to mount an anamnestic response may not be entirely satisfactory.

Further, it is easy to be too dismissive of the importance of symptomless HBV infection based on anti-HBc seroconversion in vaccine responders and nonresponders. The suggestion in an editorial [Hall, 1993] that "whether antibody responses after vaccination should be verified and subsequent decay documented will depend on local resources" is not acceptable in the interest of the public health, apart from other considerations.

An early placebo-controlled study was carried out with a plasma-derived vaccine in an HBV "high-risk" setting in 353 staff, patients on maintenance haemodialysis, and their relatives in France in 1975 [Maupas et al., 1979]. Follow-up of 73 patients and 191 staff showed that vaccinated subjects who did not respond to the vaccine by developing anti-HBs were infected at the same rate as the unvaccinated controls, i.e., nearly 50% as indicated either by anti-HBc production alone (5%), transient antigenaemia (15%), or prolonged antigenaemia (25%). Many of the subjects who developed infection within 2 months of immunisation were patients, who tend to mount a delayed or slow anti-HBs response and were likely to be incubating the infection. Thirteen staff members (60%) were nonresponders and

nine became infected with HBV within 4 to 12 months after the first inoculation.

It should be noted that interpretation of parts of the report is difficult. Other studies [Stevens et al., 1984a; Coursaget et al., 1986; Hadler et al., 1986; Couroucé et al., 1988; Taylor and Stevens, 1988] have shown that the risk of HBV infection increases as anti-HBs levels decline to 10 IU/L in responders. There are few reports concerning nonresponders. Nevertheless, the initial efficacy trials of the plasma-derived hepatitis B vaccine (produced by Merck, Sharp & Dohme in the United States) provide evidence of the continuing susceptibility of persons who receive a complete course of vaccine but develop less than 10 IU/L of anti-HBs. For example, the study conducted by Szmuness et al. [1981] revealed that 7 of 21 (33%) of vaccinated nonresponder male homosexuals became infected during an 18-month period of surveillance. That compared with 92 of 426 (22%) placebo recipients infected during the same period. The evaluation in another study of long-term protection by hepatitis B vaccine for 7–9 years revealed 36 HBV infections among 139 male homosexuals who had no detectable anti-HBs after three doses of vaccine [Hadler et al., 1991]. In an earlier trial, the same investigators noted that HBV infection occurred in 55 vaccinated subjects with a poor antibody response and 2 became carriers of HBV, both of whom were nonresponders [Hadler et al., 1986]. In another study there were 4 "vaccine failures" among 15 babies born to high-risk mothers; one infant nonresponder became infected at the age of 10 months and one poor responder became infected at the age of 6.5 months and remained *e* antigen positive for 5 months of the follow-up [Flower and Tanner, 1988].

There are apparently no reports of a cohort of healthy nonresponders to vaccination who have been surveyed systematically for a sufficient number of person-years to closely estimate susceptibility to infection. It is proposed to follow-up by serological surveillance the 86 nonresponder participants in the Hep B-3 vaccine, amongst whom the overall rate of seroconversion in terms of anti-HBs titre of >10 IU/L was 66%, with rates ranging from 55 to 76% across antigen doses of 5, 10, 20, and 40 μg [Zuckerman et al., 1996].

## NONRESPONDERS AND SILENT INFECTION

A brief report [Lou et al., 1992] noted that 6.4% of 214 subjects in China who were immunised with the Merck, Sharp & Dohme hepatitis B vaccine and 12.5% of 96 subjects who received a locally produced vaccine did not respond. HBV DNA was detected by PCR in over 60% of the nonresponders in each group, suggesting that nonresponsiveness to hepatitis B vaccine may be due to immunotolerance or immunosuppression induced by latent HBV infection. Other reports suggested that HBV *e* antigen can cause immunotolerance and chronic HBV [Brunetto et al., 1991], and that HBV itself may cause immunotolerance by infecting directly T and B lymphocytes resulting in viral persistence [Oldstone, 1989], or through different mechanisms triggered by viral infection leading to imbalance in immunoregulation [Paller and Mallory, 1991].

No evidence of latent HBV infection was found in 86 nonresponders in the study carried out by Zuckerman et al. [1996]. This observation was made after the repeated absence of serological markers of infection in the subjects (anti-HBc and HBsAg), and by the absence of HBV DNA by nested-PCR in many of the nonresponder subjects at the Royal Free Hospital and School of Medicine (Dr. T.J. Harrison, personal communication) and in four volunteers with anti-HBc.

## ATTEMPTS TO OVERCOME NONRESPONSIVENESS BY THE USE OF IMMUNOMODULATORS

Attempts have been made to enhance the anti-HBs response following immunisation, particularly in patients treated by maintenance haemodialysis, but often with conflicting results or in limited studies, which have not been confirmed: alpha interferon [Grob et al., 1984, Goldwater, 1994]; interleukin-2 [Meuer et al., 1989, Jungers et al., 1994]; and thymopentin [Zaruba et al., 1983, Melappioni et al., 1992]. Other substances such as experimental oral adjuvants in mice and oestrogen have been referred to for the sake of completion.

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